Chapter 3

Salmonella enterica: Latency

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Additional information is available at the end of the chapter

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Abstract

Infection caused by more than 1500 serotypes of *Salmonella enterica* subsp. *enterica* is one of the most common food-borne diseases, prevalent worldwide. Concerning public health, *Salmonella* latent carrier animals represent an important source of transmission of the disease. They are responsible for silent introduction of the bacteria into the food chain and the environment. Most pathogenesis studies of salmonellosis are focused on events that lead to clinical disease. Researchers have been unable to clearly discern the interaction between intracellular microorganisms and their resistant hosts in latency. However, understanding this interaction is essential for the proper employment of the control and eradication strategies. Thus, the objective of this article is to present an overview of some important events that occur during the infection cycle of *S. enterica* in latent carriers.

Keywords: *Salmonella* asymptomatic carrier animals, pathogen-host interaction, pathogenisis, public health, intracellular bacteria

1. Introduction

The genus *Salmonella* belongs to family Enterobacteriaceae, and its classification follows the Kauffmann-White scheme, which groups serotypes according to their somatic, flagellar and capsular antigens. Serotyping is essential for investigation of outbreaks of salmonellosis, contributing to epidemiological surveillance. Currently, the genus consists of two species, *S. enterica* and *S. bongori*, the first being subdivided into six subspecies, which are designed by Roman numeral, containing more than 2500 antigenically distinct serotypes. Of these serotypes, around 1500 belong to *Salmonella enterica* subspecies *enterica (I)*, which colonizes the intestinal tract of warm-blooded animals and is responsible for 99% of *Salmonella* infections, while the others pertain to other subspecies: *salamae (II)*, *arizonae (IIIa)*, *diarizonae (IIIb)*,

houtenae (IV) and *indica (VI)*. Although *S. bongori* has been determined to be a separate species, it was originally designated as subspecies V, which is commonly found in cold-blooded animals and in the environment [1–3]. After serotyping by Kauffman-White scheme, characterization by pulsed-field gel electrophoresis (PFGE) pattern and phage typing provides further subtyping [3]. Eventually complete genome sequencing will be the norm as the cost of such analysis has come down basically replacing multiple-locus variable-number tandem repeat analysis (MLVA) [4].

Most outbreaks of salmonellosis in humans and in domestic animals are caused by a few serotypes, which are grouped according to their adaptation to the host. The first group consists of a few *host-specific serotypes*, which typically cause systemic disease in a single animal species or a limited number of phylogenetically-related species. Noteworthy examples are *S. enterica* serotype Typhi and Paratyphi of humans, serotypes Pullorum and Gallinarum of birds and serotype Abortusovis of sheep. The second group consists of *host-adapted serotypes* that are associated with one or two animal species that are related to each other; however, they may occasionally cause disease in other hosts. Noteworthy examples are *S. enterica* serotype Dublin and serotype Choleraesuis, which are usually associated with severe systemic disease in ruminants and pigs, respectively. Finally, the third group consists of a large numbers of *ubiquitous serotypes*, which typically cause gastroenteritis in a wide variety of unrelated host species;among these are *S. enterica* serotype Typhimurium and serotype Enteritidis [5], and these are the two most prevalent serotypes in the world [6].

Epidemiologically, infections caused by *Salmonella enterica* subsp. *enterica* correspond to the most prevalent disease transmitted via food worldwide. This high prevalence is associated with the absence of clinical disease in animals that often silently infect herds, contaminate food, the environment and thus cause disease in humans. However, historically, studies on the pathogenesis of salmonellosis are focused on events leading to clinical manifestations, and a few studies are conducted to clarify the interaction between latent microorganisms and their resistant hosts.

Certain animal species may develop asymptomatic persistent infection with intermittent shedding of *Salmonella* in their feces over long periods. These animals are called *latent carriers*. Their impact on public health is that the carriers are natural reservoirs of different *Salmonella* serotypes and may be resistance to multiple antimicrobials. Latent *Salmonella* infections can occur in humans [7], in farm animals such as cattle, sheep, pigs and poultry [5], in pets such as dogs [8] and in wild animals such as reptiles [9, 10].

Latent carrier animals are therefore natural reservoirs of *Salmonella* and are responsible for the silent intermittent introduction of the pathogen into the food chain and the environment, hindering control strategies. Thus, increasing our knowledge regarding the interaction of intracellular pathogen *Salmonella* with their host is essential for the development of an efficient strategy for control. In this mini-review, we present some important events that occur during the infection cycle of *S. enterica* leading to latent carriers, including the mechanisms of invasion of the host cells, bacterial multiplication and persistence in intracellular compartments, and intermittent shedding of the pathogen in the feces.

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2. Pathogenesis of *Salmonella enterica*: the role of *Salmonella* pathogenicity islands (SPIs)

The pathogenesis of salmonellosis depends on a combination of several factors, including the components of bacterial virulence, the infective dose, route of infection, the genetic makeup and the immune status of the host [11]. All of these variables can influence the immunological responses of the host, resulting in different degrees of inflammation that confer an acute, moderate, chronic or even asymptomatic nature to the disease [12].

Infection by *S. enterica* has the following characteristics: the ability to interact with enterocytes leading to diarrhea (*Salmonella*-induced enteritis), the invasion of non-phagocytic cells and the ability to survive and proliferate within the phagocytes, resulting in systemic disease [13]. These characteristics are determined by multiple virulence factors encoded in *Salmonella* pathogenicity islands (SPIs) comprising large and unstable segments of the bacterial genome of pathogenic organisms. These SPIs are absent in related non-pathogenic organisms and that were acquired by horizontal gene transfer as SPIs G + C content is lower than *Salmonella* genes [14]. SPIs are conserved in several strains; differences may have implications in host specificity [15]. Currently, 16 pathogenicity island of *Salmonella* encoding distinct virulence factors are described, according to pathogenicity island database, PAI DB (http://www.paidb.re.kr), with different distributions among the various *Salmonella* species, subspecies and serotypes. SPI-1 and SPI-2 (both are about 40 kb in length) are the most studied and are present in all subspecies of *S. enterica* [13, 14, 16]. SPI-1 contains the genes responsible for the bacterial invasion of the host epithelium [17, 18], whereas SPI-2 is responsible for bacterial survival and multiplication within eukaryotic cells, including macrophages [19, 20].

Studies of SPIs help in understanding the mechanisms of bacterial virulence, and they may also be useful to clarify the phylogenetic relationships among species [21, 22]. Phylogenetic studies indicated that the gene sequences present in SPI-1 were acquired by lateral gene transfer before the diversification between *S. enterica* and *S. bongori*. In turn, the acquisition of the SPI-2 genes present in *S. enterica* occurred after speciation but before the diversification of the groups (I, II, IIIa, IIIb, IV, VI and VII); therefore, SPI-2 is present in all *S. enterica* subspecies but is absent in *S. bongori* species [22].

The virulence mechanisms of *Salmonella* serotypes are studied in different animal models, depending on the type of clinical manifestation. To study the pathogenesis of typhoid fever (a systemic disease), strains of susceptible mice (*e.g.*, Balb/c) experimentally infected with serotype Typhimurium are used. However, in this experimental model, the mice do not develop diarrhea, and therefore, mice are not used to study the pathogenesis of enteritis. In contrast, the experimental infection of calves with the same serotype results in enteric disease, and therefore, this experimental model is used to study *Salmonella*-induced enteritis [23].

According to the animal model, the virulence genes required for systemic infection differ from those genes responsible for the enteritis caused by *Salmonella*. This result is observed by analyzing mutant phenotypes of serotype Typhimurium in experimental infection of mice and calves, which are used to study systemic and enteric infections, respectively. Mutations in

SPI-2 result in a significant attenuation of systemic disease in mice, while in calves, the severity of intestinal lesions shows only modest attenuation. In contrast, mutations that prevent the expression of the SPI-1 type III secretion system (T3SS) or of effector proteins translocated by the system result in an avirulent strain with consequent the absence of diarrhea in calves [23].

2.1. SPI-1-mediated invasion of host cells

After oral infection, a proportion of the *Salmonella* organisms survives the low stomach pH and reaches the distal ileum and cecum, where they invade the epithelial cells and M cells, mediated by a T3SS encoded by the SPI-1 [24, 25]. The T3SS allows some of the enteropathogenic bacteria to adhere to the epithelial surface and inject effector proteins that cross the membrane of the host cells, causing cellular injury [26]. Through this system, *Salmonella* translocates effector proteins encoded by genes present in the SPI-1 as well as genes in independent *loci* of the SPI-1 that promote a chain of events in the host cell to allow pathogen invasion [13]. Another function of the SPI-1 is related to hydroelectrolyte imbalance caused by the effector protein SopB, which stimulates the secretion of chloride ions (Cl^-) through its inositol phosphatase activity, thereby leading to loss of fluid into the intestinal lumen [27] (**Figure 1**).

Once in contact with the intestinal epithelium, the effector proteins SopE, SopE2 and SopB (encoded by genes outside of SPI-1) are translocated to the interiors of enterocytes and M cells via the SPI-1 T3SS. These proteins activate certain GTPases within the host cell, such as Cdc42, Rac-1 and Rho, causing a rearrangement of the actin cytoskeleton called membrane ruffling [28], which is stabilized by the SipA and SipC effector proteins. Furthermore, they also activate the MAP kinase (mitogen-activated protein kinase) pathway, thereby destabilizing tight junctions. Consequently, bacteria can penetrate into the host cell through the apical membrane in a process called macropinocytosis or cross the intercellular space until reaching the lamina propria. This destabilization of tight junctions also allows for the transmigration of polymorphonuclear cells (PMNs) from the basolateral space to the apical surface. However, this transmigration can occur independently from the destabilization of tight junctions when mediated by the bacterial protein SopA [29]. Once inside the cell, the effector protein SptP modulates the inactivation of the GTPases Cdc42 and Rac-1, thus resulting in the end of the membrane ruffling [30].

Signaling via MAP kinase, in addition to promoting the destabilization of tight junctions, also activates the transcription factors AP-1 (activator protein-1) and NF-κB (nuclear factor- κB), which leads to the synthesis of pro-inflammatory interleukin (IL)-8 by PMN leukocytes, thus acting as a chemotactic factor for neutrophils [29].

During the invasion of macrophages, the bacterium injects the effector protein SipB, which is encoded by SPI-1, inducing the intracellular activation of caspase-1 by resident macrophages. Caspase-1 induces apoptosis of infected macrophages resulting in *Salmonella* escape from these cells. Caspase-1 also cleaves the pro-inflammatory cytokines IL-1β and IL-18 to produce bioactive cytokines that further enhance the local inflammatory response, causing infiltration by PMN phagocytes and internalization of the bacterium by these cells [31, 32]. The intracellular medium provides a favorable environment for the bacteria to multiply [33], and once the invasion process is concluded, the bacteria are transported from the gastrointestinal tract to systemic organs.

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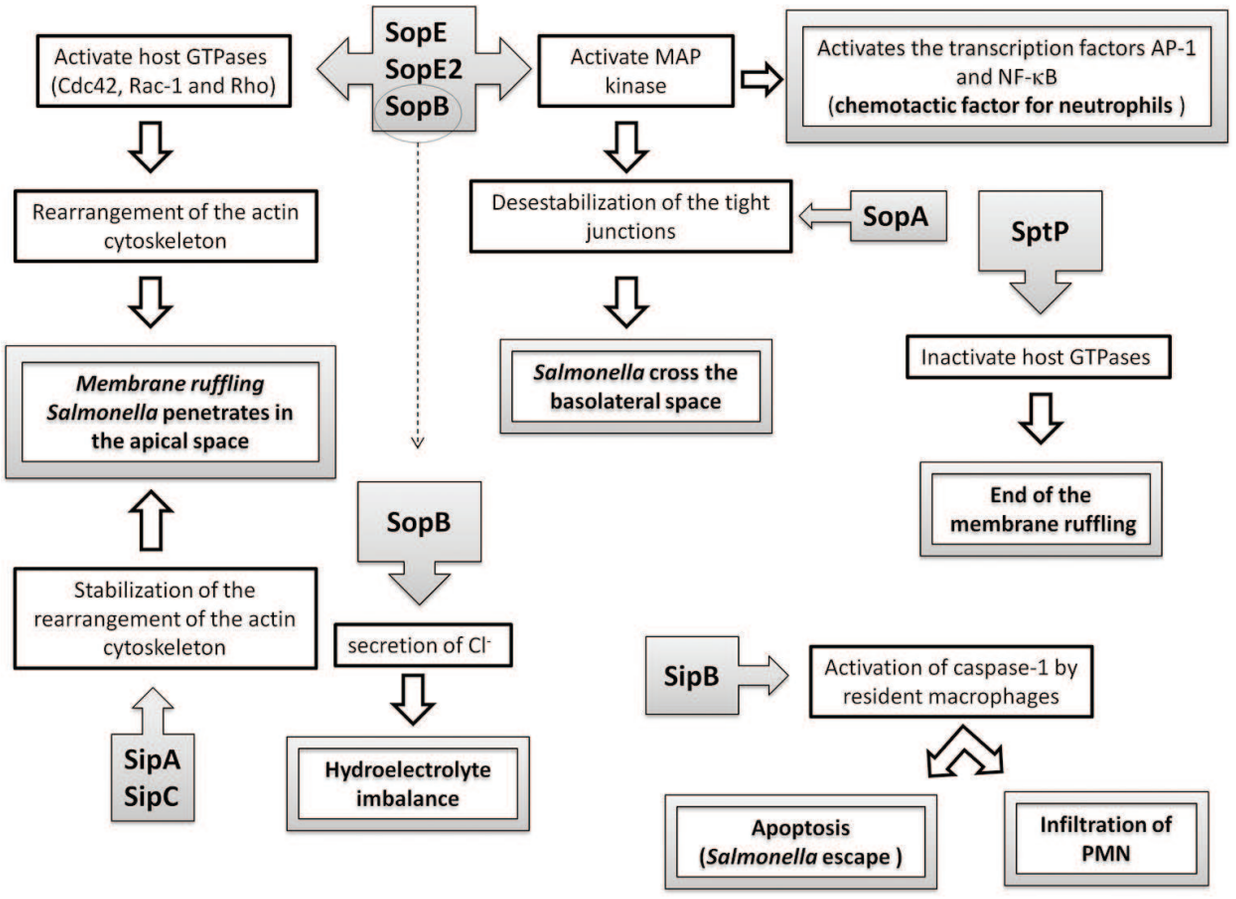

Figure 1. Effector proteins (gray arrows) ejected by type III secretion system encoded in SPI-1 and their actions for *Salmonella* invasion of host cells. *Salmonella* penetrates at the apical space causing the membrane ruffling. It is mediated by SopE, SopE2 and SopB proteins, which promote activation of host GTPases, causing a rearrangement of the actin cytoskeleton that is stabilized by SipA and SipC proteins. *Salmonella* can also cross the basolateral space through destabilization of the tight junctions, also mediated by SopE, SopE2 and SopB proteins (by activation of MAP kinase pathway) and by SopA protein. These events contribute to the activation of chemotactic factors of neutrophils. Once inside the cell, *Salmonella* promotes the end of the membrane ruffling by inactivation of host GTPases by SptP protein. During the invasion of resident macrophages, SipB protein induces the intracellular activation of caspase-1, causing apoptosis and enhancing the local inflammatory response. This event contributes to the escape of *Salmonella* from the macrophages and internalization of the bacteria in PMN phagocytes. The hydroelectrolyte imbalance is caused by SopB protein through inositol phosphatase activity which stimulates the secretion of chloride ions (Cl^-).

There is an alternative SPI-1-independent invasion mechanism in which *S. enterica* does not interact with M cells but is engulfed by dendritic cells that open the tight junctions between epithelial cells, thereby carrying the bacteria to systemic organs [34].

2.2. SPI-2-mediated intracellular multiplication

The ability of *S. enterica* to survive inside phagocytes and to replicate in *Salmonella*-containing vacuoles (SCV) in a variety of eukaryotic cells is dependent on another T3SS that is encoded by SPI-2 [22, 35, 36]. This characteristic can lead to systemic infection [20].

Soon after entry by means of macropinocytosis, *Salmonella* is internalized into a phagosome formed by the membrane ruffling that later fuses with lysosomes, thereby originating the SCV

[29]. Inside of the SCV, the T3SS encoded by SPI-2 is activated using luminal acid pH, translocating the effector proteins across the phagosome membrane (**Figure 2**). The effector protein SipC prevents the fusion of the SCV with vesicles containing NADPH oxidase (nicotinamide adenine dinucleotide phosphate-oxidase) and inducible nitric oxide synthase (iNOS), hindering the action of reactive oxygen intermediates (ROS) and reactive nitrogen intermediates (RNS) [13]. The effector proteins SifA and PipB2 contribute to the formation of *Salmonella*-induced filaments (SIF) along microtubules, while the effector proteins SseF and SseG aggregate the SCV-adjacent microtubules. In addition, an accumulation of actin occurs around the SCV that is mediated by the SspH2, SpvB and SseI proteins. These events contribute to the maturation and stabilization of SCV [29]. As a consequence, *S. enterica* becomes even more protected against RNS and ROS and against the potent antimicrobial activity of peroxynitrite, which is generated by the RNS and ROS reactions. These mechanisms represent a specific adaptation of *S. enterica* to the intracellular environment, especially phagocytes. Thus, the bacteria can multiply inside the phagocytic cells, transported via circulation and cause systemic infection [14].

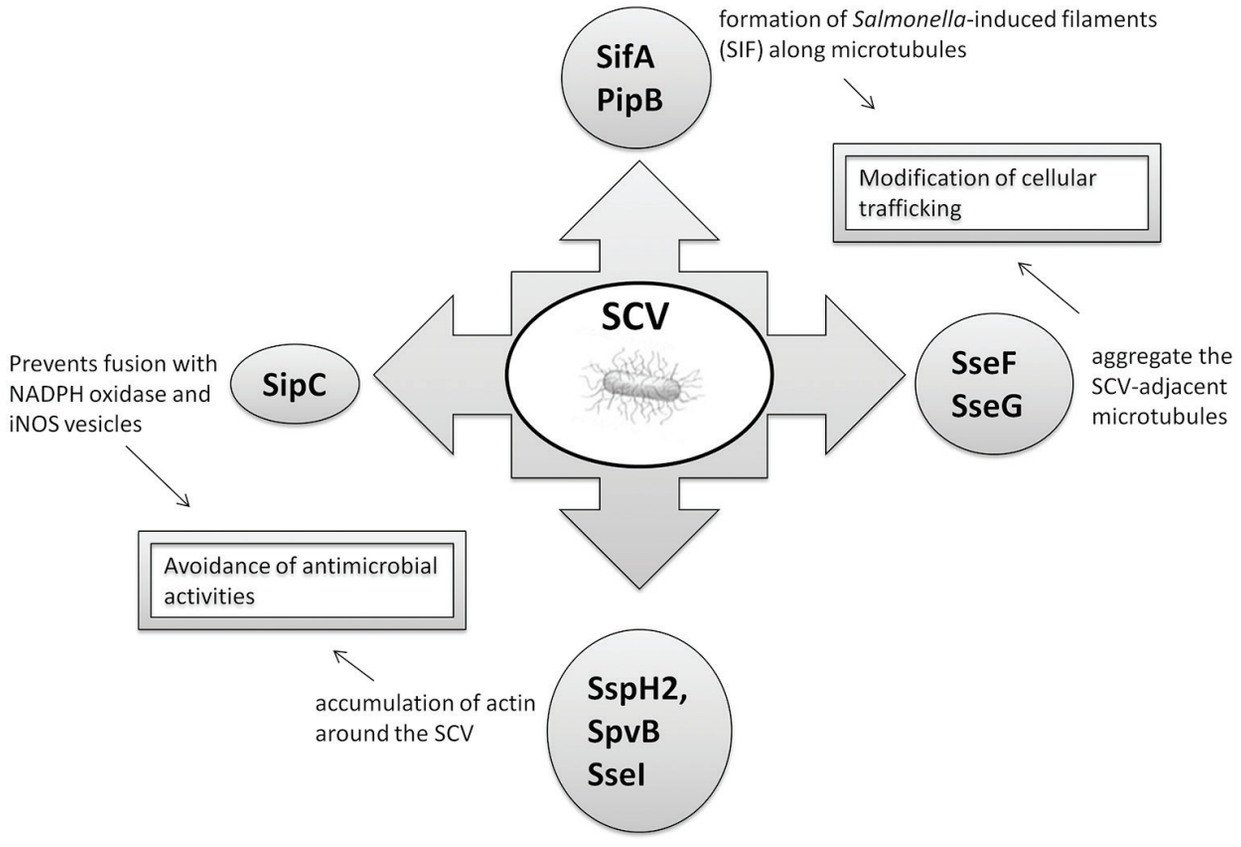

Figure 2. Effector proteins (gray circles) ejected by type III secretion system encoded in SPI-2 and their actions for *Salmonella* survival inside of phagocytes and its replication in *Salmonella*-containing vacuoles (SCV). The translocation of SipC protein avoids the antimicrobial activities of reactive oxygen intermediates and reactive nitrogen intermediates by prevention of fusion of NADPH oxidase and iNOS vesicles. This antimicrobial activity by the host cell is stronger but prevented by the accumulation of actin around the SCV promoted by SspH2, SpvB and SseI proteins. These events contribute to maturation of SCV. SifA and PipA proteins contribute to the tubular structures known as *Salmoniella*-induced filaments formed along the microtubule motors; in addition, SseF and SseG cause microtubules aggregation adjacent to SCV. These events interfere the molecular motors that drive the cellular trafficking, which transport vesicles and organelles within the cell.

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3. Natural resistance mechanism to infection by *S. enterica*: the role of Nramp1 glycoprotein

The resistance mechanisms of host to infection by *S. enterica* are multigenic. Studies in mice have emphasized the *locus* encoding glycoprotein natural resistance-associated macrophage protein-1 (Nramp1), which has been considered the key for the innate host response to intracellular pathogens [37]. This protein belongs to a family of proteins highly conserved in evolution, with homology among mammals, insects and bacteria suggesting an important role in all living organisms [38].

Nramp1 is a transmembrane glycoprotein and divalent metal ion symporter that deprives intracellular pathogens of these metals by removing mainly Fe^{++} and Mn^{++} from the luminal space of the phagosomal and lysosomal vesicles. Because iron and other divalent cations are cofactors for vital enzymes, *S. enterica* expresses a series of carriers that compete with the host cell for traces of these divalent metals within the phagosomes [39]. This Nramp1 glycoprotein is encoded by the gene *Slc11a1* (*Solute carrier family 11 member 1*, first named as *Ity* gene), on chromosome 1 in mice [37]. A single substitution of glycine for aspartate at position 169 results in susceptibility to systemic infection by *S. enterica* in the mice [40]. Consequently, mice that have two *Slc11a1* $^{Asp\ 169}$ alleles are significantly more susceptible to lethal *Salmonella* infections and are therefore being used in studies to clarify the host-pathogen relationships in acute systemic infection. In turn, mice carrying the wild-type *locus* $Slc11a1^{+/+}$ can be used to study the pathogenesis of chronic infections that are often asymptomatic [41].

The interaction between the surface receptors of macrophages and microbial ligands results in the internalization of the microorganism into a phagosome. However, this young phagosome is not able to digest its contents, thus requiring a maturation process involving fusion and fission events with endosomes and lysosomes. During the maturation process, phagosomes containing *S. enterica* acquire vacuolar ATPases that acidify the phagosome lumen. In an acidic pH, Nramp1 removes Fe^{++} and other divalent cations from the inside of phagosomes. Concomitantly, in the presence of functional protein Nramp1, the host cell expresses the mannose-6-phosphate receptor (M6PR), which is responsible for interacting with vesicles containing NADPH oxidase and iNOS. This interaction generates positive feedback for the transcription of high levels of iNOS mRNA [39]. In susceptible mice ($Slc11a1^{Asp\ 169}$), the phagosomes containing *S. enterica* are negative for M6PR receptors, and therefore, the production of iNOS is lower than in hosts that have the wild-type *locus* $Slc11a1^{+/+}$ [36]. Thus, Nramp1 has proven to be very important to control the exponential growth of *Salmonella* during the early stages of systemic infection [23, 42].

4. Infection cycle of *S. enterica* in latent carriers

In asymptomatic carrier animals, the study of the infection cycle of *Salmonella* was described using C57Bl/6-Bcgr ($Slc11a1^{+/+}$) mice as a resistant mouse model inoculated orally with a high dose of *Salmonella* serotype Enteritidis [43]. The animals developed an intermittent infection cycle in the gastrointestinal tract during 4 weeks of study, with interspersed periods of

intra- and extracellular spread of the infection, which featured three distinct stages over the course of the cycle (**Figure 3**): *(I) the initial stage* represented by intracellular invasion and bacterial multiplication in the intestine, inducing transient damage to the intestinal mucosa and shedding of the pathogen in the feces. A rapid clearance of a large fraction of the inoculums was observed during the first 48 h postinoculation (PI); (*II) the intermediate stage*, the initial period of bacterial sequestration by the mononuclear phagocyte system (MPS) in which the pathogen was detected only within intracellular compartments. In this period, a transient exponential growth of the remaining intracellular bacteria occurred 2–4 d PI followed by a suppression of bacterial growth, establishing a plateau phase until 15 d PI. The intracellular multiplication in the MPS coincided with the IFNγ production; and finally (*III) the intermittent shedding stage*, the *Salmonella* persists sub-clinically in the tissues (spleen and cecum) with recurrence of intracellular bacterial growth that coincided with the intermittent excretion in feces, characterizing a latent infection.

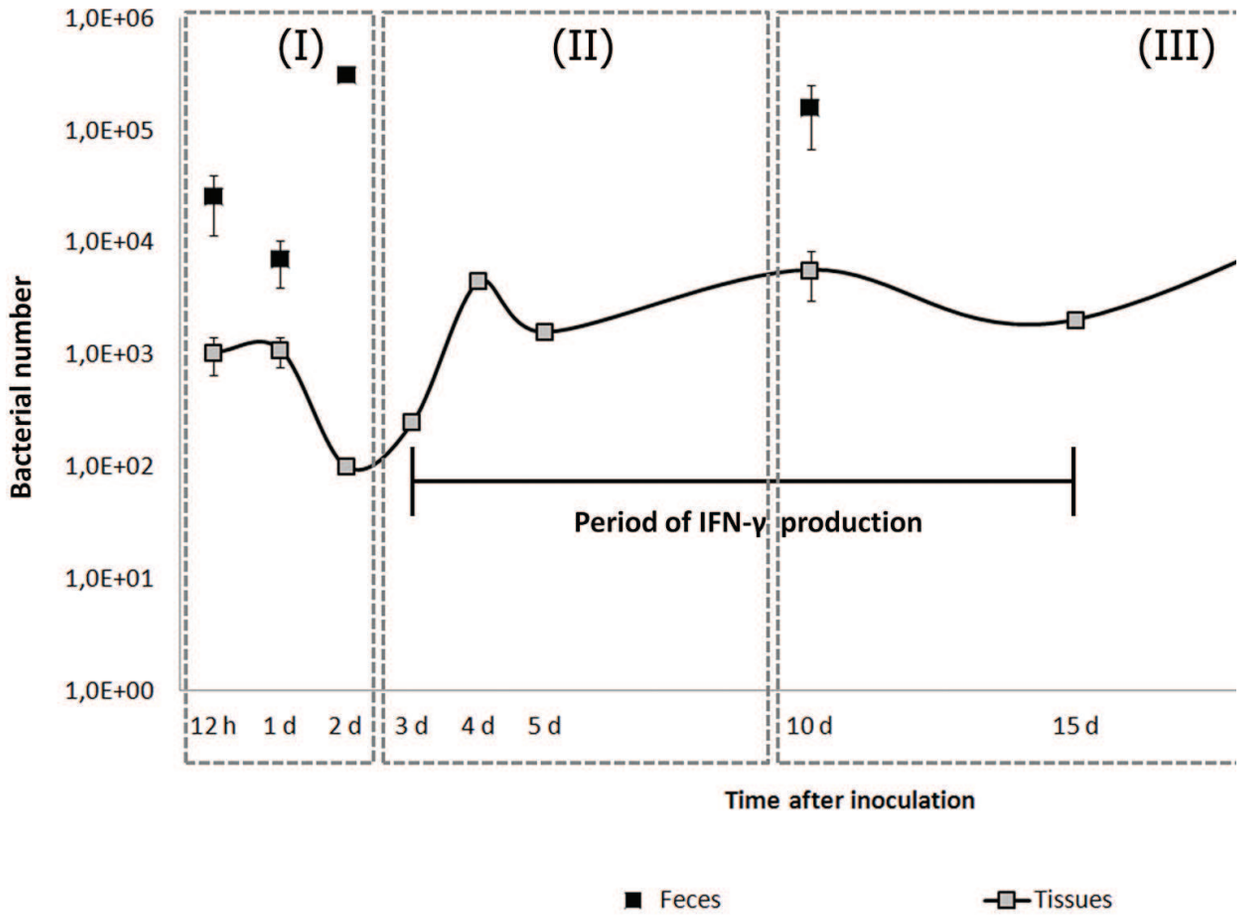

Figure 3. Distribution of *S. enteritidis* in feces (fecal and ileo-cecal content) and tissues (blood, spleen, liver, mesenteric lymph nodes and different parts of the intestine—jejunum, ileum and cecum) at different times after intragastric inoculation with 5 × 108 cfu in C57Bl/6-Bcgr (Slc11a1$^{+/+}$) mice as a resistant mouse model. These numbers are represented as mean ± SD of three animals (in duplicate). (I) Initial stage of infection, when *Salmonella* invades the intestinal mucosa and it is also eliminated in feces. (II) Intermediate stage marks the initial period of mononuclear phagocyte system (MPS) sequestration. *Salmonella* is found intracellular in the intestine but it is not being eliminated to the environment through feces. (III) Intermittent elimination stage of *Salmonella*, common in a resistant animal model, based on [43].

In pigs, by applying a Markov statistical model, Ivanek et al. [44] were able to distinguish different stages during the dynamic shedding of *Salmonella* in feces and their immune response. In this model, the intermittent shedding of the pathogen was clear. The authors characterized the following stages: (i) *latency*, when pigs were negative for the shedding of *Salmonella* immediately after the challenge; (ii) *continuous shedding*, with continuous shedding of the pathogen in the feces; (iii) *non-intermittent shedding*—when *Salmonella* was not being shed in the feces; (iv) *intermittent shedding*—when the bacteria were again shed in the feces; and (v) *recovery*. The authors observed that the stages could vary depending on the infecting dose and the serotype involved in the infection.

Thus, independent of the animal model, in latent carriers, there is a period during which *Salmonella* stays hidden in an intracellular compartment, and it is not being eliminated. It can mask the diagnosis of the positive animals. This "*Salmonella*'s hiding-place" may function as a strategic site of bacteria multiplication and, consequently, elimination of high numbers of pathogens in the environment. So, it is very important to identify the sites of bacterial colonization in different latent carriers.

The site of bacterial colonization in persistent infections varies according to serotype and host species. In humans, serotype Typhi expresses proteins encoded by SPI-7 that inhibit the detection of pathogens by the innate immune system of the host. Thus, the bacteria can spread systemically, colonizing macrophages in the liver, spleen and bone marrow. In the liver, *Salmonella* serotype Typhi can be found latent in the gallbladder, making the host an asymptomatic carrier. Intermittently, the bacteria are transported from the gallbladder into the small intestine through the bile and excreted in the feces [7]. In mice, the mesenteric lymph nodes are the colonization site of serotype Typhimurium [41]. In birds, *Salmonella* serotype Pullorum can be found latent in the spleen, ovary and oviduct of chickens [45], and *S*. Enteritidis can infect the ovaries of healthy hens, contaminating the eggs prior to shell formation [46]. In snakes, there is strong evidence that different serotypes of *Salmonella* also colonize the ovary, spreading bacteria to their offspring vertically [47].

In asymptomatic animals, the cecum plays an important role as a reservoir for longer periods of shedding [48–51]. Research using resistant mice orally challenged with high doses of *Salmonella* serotype Enteritidis [43], and we demonstrate that bacteria reach the cecum in the early stages of infection (12 h to 2 days PI) and remain for long periods from 5 days PI, functioning as a reservoir of bacterial multiplication, causing the shedding of *Salmonella* in the intestinal lumen intermittently. The small intestine does not have this reservoir role, since the bacterial colonization in jejune and ileum occurred only in 1–4 days PI. Spleen is another site of *Salmonella* reservoir; from the moment that bacteria reached the MPS, they stayed in spleen for long periods (**Figure 4**).

In chickens, the cecum is also a site for long-lasting carriage of *S*. Enteritidis, both in susceptible and resistant animals [52]. In asymptomatic carriers, it represents a public health and food protection concerns because the cecum may function as a "strategic site" of *Salmonella* proliferation, releasing bacteria to the environment intermittently.

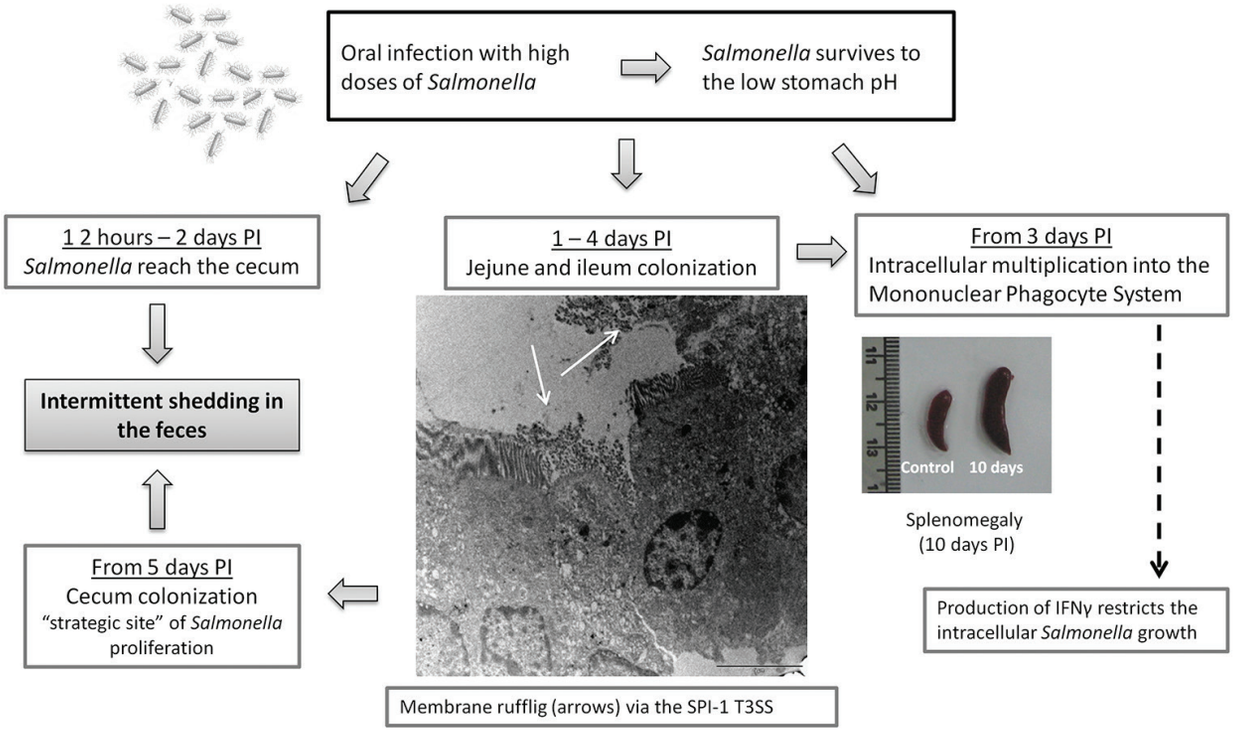

Figure 4. Course of *S. enteritidis* in C57Bl/6-Bcgr (Slc11a1$^{+/+}$), a resistant mouse model. *Salmonella* rapidly reaches the cecum in the early stage of the infection between 12 and 48 h postinoculation (PI) and remains in this organ as an important reservoir for 5 days PI, with increasing bacteria multiplication. The presence of bacteria in the cecum seems to be associated with its extracellular multiplication in the intestinal content and intermittent shedding in the feces. The colonization of the small intestine occurs during the first 4 days PI. In this period, *Salmonella* penetrates the intestinal mucosa, causing different degrees of degeneration of the microvilli, which is reversible (membrane ruffling). This mechanism is mediated by effector proteins translocated by T3SS encoded in SPI-1. Intracellular multiplication of the bacteria in mononuclear phagocyte system (MPS) occurs from 3 days PI. The exact route of *Salmonella* dissemination from intestine to MPS is unclear, but from the moment that bacteria reach the MPS, they remain in spleen, causing splenomegaly by 10 days PI. The intracellular multiplication in MPS coincides with the production of IFN_{γ}, which restricts the replication of intracellular *Salmonella*.

The mechanism of persistence of *Salmonella* in the cecum is not well established. Probably, it is associated with the physiological environment and less peristalsis of this part of the intestine. Upon entry into the large intestine, the bacteria remain longer in the cecum due to fewer peristaltic movements. Despite the production of short-chain fatty acids by resident microbiota due to the intense local fermentation, the pH in the cecal environment remains above 6.3, higher than the inhibitory level for *Salmonella* multiplication [53].

5. Role of IFNγ in controlling of *S. enterica* growth

During intestinal infection, *Salmonella*-host interactions result in different degrees of inflammation related to the levels of cytokines produced [12], which may trigger changes in the composition of the intestinal microbiota. A reduction in symbionts or an increase in pathobionts is usually observed during inflammatory processes, reflecting the diversity of the intestinal microbiota [54]. In gastroenteritis caused by *Salmonella* in susceptible hosts, the production of interferon gamma (IFNγ) in the early stage of intestinal inflammation may alter the

lumen conditions, causing an imbalance in the ecology of the resident microbiota that favors competition for pathogen growth and intestinal colonization [55–57]. In latent carriers, however, *S. enterica* can invade the intestinal mucosa and colonize the intestine without triggering a strong immune response, remaining in equilibrium with the resident microbiota [58].

IFNγ plays a crucial role in resistance to systemic infection by *S. enterica*. This cytokine controls the growth of pathogens both in the initial [59, 60] and late stages of the disease [41], and its absence results in septicemia. High levels of IFNγ as well as of its mediator IL-12 contribute to resistance to infection in different animal species [61]. Mice with chronic asymptomatic infection by *Salmonella* serotype Typhimurium develop symptoms after treatment with anti-IFNγ antibodies [41]. In birds, the IFNγ gene expression is lower in susceptible animals than in resistant animals [61].

IFNγ is produced specifically in response to systemic infection and correlates with bacteremia and pathogen invasion of the cells of the mononuclear phagocyte system, such as the lymphoid tissue associated with the intestine (mesenteric lymph nodes and Peyer's patches), spleen and liver. Its production is essential to restrict bacterial intracellular multiplication, thereby contributing to the establishment of a plateau phase during the growth cycle of *Salmonella* serotype Enteritidis in asymptomatic mice [43].

When antigen-specific acquired immunity is triggered, the IFNγ titer in serum begins to decrease [11]. However, even in the presence of high titers of specific circulating antibodies, some *Salmonella* serotypes are capable of causing persistent infections in a host for long periods. This adaptive immune response seems to be important to reduce the number of extracellular bacteria; however, bacteria that are present within macrophages survive both the innate and adaptive immune responses, and the host ultimately becomes a latent carrier [41].

6. Gene expression in latent *Salmonella*

Zoonotic intracellular pathogens that can cause latent carriers pose a unique public health problem. The ability of such carrier animals to shed pathogens without showing any clinical signs of infection can make outbreak control challenging and the potential of transmission to humans a serious public health concern. Before identifying these carriers, we need to understand the mechanism of bacterial invasion of the host cells and follow the process of establishing a persistent state of infection. SPI 1 encodes for genes *hil*A and *inv*F, which allow the bacteria to enter, survive, and replicate within the host cells [62]. Once the pathogen enters the host cells, glycine cleavage protein subunit P (*gcv*P) has been shown to be a potential key player in the transition from acute to chronic infection [63–65]. The activity of *gcv*P has been shown to increase dramatically in other important zoonotic infections like tuberculosis [66, 67] and leishmaniasis [68]. Understanding the pathogenesis of the invasion, intracellular replication, and the transition to latent carrier state in *Salmonella* would potentially lay the groundwork for the development of a control, treatment and eventual eradication strategies. We are just starting to understand potential genes involved in the transition from active to latent stage of infection in case of intracellular pathogens. There is very little information in case of *Salmonella*, but in case of *M. tuberculosis*, glycine dehydrogenase activity increases

tenfold upon entering a state of persistence. Another indicator that its metabolism is vital to persistence is the fact that mutants that are deficient in isocitrate lyase, an enzyme involved in the glyoxylate pathway, cannot cause chronic latent infections [67]. We have some preliminary results from our long-term cell culture *Salmonella* infection model (unpublished personal communication). It shows that *Ace*A the gene that codes for isocitrate lyase, which is the first step in the glyoxylate shunt, is over expressed. Even on day 1, the expression levels are elevated, but not significantly more than any of the other genes. However, on day 10 and day 30 post infection,. *Ace*A expression level on day 30 goes up dramatically. This has biological plausibility since it is the first step in the glyoxylate pathway. Such gene expression studies of lymph node biopsies on a herd basis or at slaughter might allow us to detect chronic/persistent *Salmonella* infections.

7. Conclusions

Despite host's activation of anti-inflammatory and antimicrobial responses, *Salmonella* can establish asymptomatic persistent infections, leading to intermittent high-level shedding of the bacteria in feces. This host-pathogen balance leads to serious problems for public health because asymptomatic animals latently carry the infection for long periods with intermittent cycles of shedding of the pathogen in feces. This outcome is epidemiologically important because false-negative *Salmonella* isolation results can be generated if the diagnostic test is performed during the period when the animal is not shedding the pathogen.

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